Reference: *Biol. Bull.* **163**: 492–503. (December 1982)

# MEMBRANE-STABILIZING AND CALCIUM-BLOCKING AGENTS AFFECT *ARBACIA* SPERM MOTILITY

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## ABSTRACT

The speed and duration of progressive motility of *Arbacia* sperm cells depend on the calcium content of the suspension medium. Suspended in filtered sea water (FSW) the spermatozoa undergo a progressive decline in motility (after an initial burst of activity) and cease forward movement within 30–40 min. When sperm are diluted in chemically defined artificial sea water (MBL-ASW), motility rose to about 160% of the control rate in 30 min and then gradually returned to the initial control level where it persisted for at least 40 min more. Procaine, propranolol, ouabain, and quinidine, tested singly or in combination, affected sperm motility in both time- and concentration-dependent fashion.

Procaine at 10 and 100 $\mu M/l$ in MBL-ASW caused more than a doubling in motility over the control rate, while in FSW both these concentrations were inhibitory. In FSW, quinidine had relatively little effect, while propranolol was slightly stimulatory at $10^{-6}$ $M$ and inhibitory at 0.1 and $1.0 \times 10^{-3}$ $M$. In combination, propranolol and quinidine can cause a sharp rise in motility. Ouabain increased motility dramatically in MBL-ASW suspensions. The effects of some of the drugs depend on the ability to displace calcium from binding sites in sperm cell membranes; ouabain appears to interface with Ca efflux.

## INTRODUCTION

Receptor activation and membrane lability play critical roles in the activity of many cell types. For example, induction of platelet aggregation by specific agonists is inhibited by substances classified as local anesthetics and antiarrhythmic agents; calcium antagonizes these inhibitory actions (Anderson *et al.*, 1981). Further, the effects of $Ca^{2+}$ in the medium on ciliary beat reversal in paramecium has been amply documented (Murakami and Eckert, 1972).

Similarly the movement of the mature spermatozoa of mammals and marine invertebrates is greatly influenced by interactions between sperm cell components and environmental factors. Responsiveness of sperm cell receptors to ligands, activators, and inhibitors, appears to vary with the condition of the sperm cell, its state of dilution (Gray, 1928; Rothschild, 1953), maturation (Babcock *et al.*, 1979), aging (Dunham *et al.*, 1982), capacitation, and even proximity to the ovum (Yanagimachi, 1970).

In the presence of some agents, other conditions being equal, the rate of sperm cell propulsion increases considerably. This implies that, under usual circumstances, not all of the sperm cells in a given sample are progressing at their maximal speed;

Received 28 January 1982; accepted 7 September 1982.

Abbreviations: EDTA, ethylene diamine tetra acetate; EGTA, ethylene glycol bisaminoethyltetra acetate; FSW, filtered sea water; MBL-ASW, Marine Biological Laboratory formulated artificial sea water.

This work supported by NSF Grant #PCM8002358.

that is, there seems to be a margin of safety which may permit the conservation of energy stores or otherwise enhance the union of physiologically uncompromised gametes. The difference between the optimum and the maximum swim speed capacity suggests the presence in the sperm cell of a regulatory mechanism modulated by control of calcium entry and transport through the cell as proposed here.

Procaine, added to sea water suspensions of *Arbacia* sperm, caused a rapid rise in their mean rate of forward motion followed by a sharp decline (Nelson, 1972). The local anesthetic apparently occupied binding sites in the plasma membrane, and having driven some of the bound calcium into the cell interior, then prevented its efflux.

The critical role of $Ca^{2+}$ in the modulation of sperm motility was further emphasized in studies with $ZnCl_2$, $MnCl_2$, and EDTA (Young and Nelson, 1974a) and $CaCl_2$, $LaCl_3$, and EGTA (Young and Nelson, 1974b). $Zn^{2+}$ and $Ca^{2+}$ had distinctly biphasic effects, while $Mn^{2+}$ and $La^{3+}$ as well as EDTA and EGTA were inhibitory or ineffective in the concentration ranges tested.

Cholinergic mediation appears to be involved in regulation of the entry of calcium into the sperm cell through specific ion channels (Nelson *et al.*, 1980). That is, calcium transport seems to depend on acetylcholine-induced conformational changes in a receptor channel complex that extends through the plasma membrane, similar to that proposed by Cohen and Changeux (1975) for cationic transport at myoneural junctions and electroplaques.

*Arbacia* sperm cells respond to nicotine, maximum stimulation occurring at $10^{-9}$ *M* and inhibition commencing at $10^{-6}$ *M*. The highly selective nicotinic receptor blocker, $\alpha$-bungarotoxin, completely inhibits all the cells in a suspension of *Arbacia* sperm at less than $10^{-6}$ *M*; microscopic examination showed that individual cells ceased moving at a concentration of less than 1 picomole/l (Nelson, 1976).

In mammalian sperm catecholamine sensitivity appears at the onset of the cytostructural and permeability changes coincident with capacitation (Bavister *et al.*, 1976; Cornett and Meizel, 1978), but neither epinephrine nor norepinephrine was observed to exert any appreciable change in the swim speed of *Arbacia* sperm that had not been exposed to capacitating conditions (unpublished observations).

Local anesthetics that block nerve conduction and have pronounced effects on muscle contraction raise the threshold for osmotic hemolysis of erythrocytes and interfere with platelet aggregation. The action of procaine on *Arbacia* spermatozoa attests to excitability as a physiological characteristic of the regulatory processes governing the movement of these cells.

This report extends studies on the effects of procaine on sea urchin sperm (Nelson, 1972) to include an examination of the action of the $\beta$-adrenergic blocking agent propranolol and the $\alpha$-blocker quinidine which when applied directly to cardiac muscle exerts an action similar to that of procaine. Propranolol is effective in combatting cardiac glycoside intoxication, and so the interactive effects of propranolol and ouabain on sperm motility were also examined.

## Materials and Methods

Semen was collected daily from mature *Arbacia punctulata* induced to spawn by injection of 1 ml of 0.5 *M* KCl through the oral surface of the animal. The sea urchins were inverted over 30-ml beakers filled with either filtered sea water (FSW) or chemically defined Marine Biological Laboratory artificial sea water (MBL-ASW). (The dense semen streams settled rapidly and coherently to the bottom of the beaker without dispersing). This procedure permits the preparation of samples from the

same sea urchin for suspension in either FSW or MBL-ASW, for use for an entire series of experimental runs. The supernatant fluid was decanted and the concentrated sperm cells were aspirated and transferred by means of disposable Pasteur pipettes into test tubes kept in an ice bath for the day's tests. As needed, sufficient concentrated sperm was diluted in 25 ml of FSW or MBL-ASW to yield an optical density reading between 0.500 and 0.700 in a Turner Model 350 Spectrophotometer ($\lambda$ = 480 nm), equivalent to 7–10 $\times$ $10^6$ sperm/ml (Nelson, 1972).

For each experiment, different concentrations of the test reagents were quantitatively added by micropipet to 6 separate round cuvettes and the volume brought to 0.5 ml with FSW or MBL-ASW. The tests were initiated by addition of 2.0 ml of the sperm suspension diluted immediately prior to the start of each run. The "zero-time" reading was taken in the spectrophotometer after first mixing the cuvette contents by twice inverting the parafilm-covered tube. The cuvettes were then put into the six-place horizontal rotor of an I.E.C. clinical centrifuge and spun for 4 minutes at 120 $\times$ *g* (940 rpm); this has empirically been shown to align the spermatozoa with only minimal centrifugal sedimentation of non-motile cells (*ibid.*).

Orientation of the spermatozoa subjected to low centrifugal force permits reproducible measurement of changes in optical density of the suspensions as the cells swim past the light path. As the cells are stimulated, depressed, or unaffected by varying concentrations of a given combination of agents, optical density differences between the untreated controls and the treated suspensions are recorded from the spectrophotometer. The difference in O.D. between the zero time and 4-min centrifugal runs of the various specimens (after correction for displacement of formalin-killed cells, if any, is made) is determined. All the tubes in that series are normalized to the 4-min control reference point, as percent of control motility. Motility refers to progressive motion. (For full details, see Nelson, 1972.) All of the test reagents employed—procaine (free base); ouabain $\cdot$ $8H_2O$; DL-propranolol $\cdot$ HCl, and quinidine $\cdot$ $SO_4$—were of the purest grade available from Sigma Chemical Company. Artificial sea water (MBL-ASW) prepared in the Chemical Department of the Marine Biological Laboratory, Woods Hole, MA, contained (in m*M* per liter of deionized water): NaCl, 423; KCl, 9.0; $CaCl_2 \cdot 2H_2O$, 9.27; $MgCl_2$, 22.94; $MgSO_4$, 25.50; just prior to use 0.18 mg $NaHCO_3$/l was added. The inorganic salts were of analytical reagent grade, meeting ACS specifications.

All experiments were conducted at room temperature which ranged from 22.5° to 25°C during the course of the season.

## Results

This group of test agents was selected because $Ca^{2+}$ has been implicated as a second messenger in cellular responses to their action. The swimming capacity of *Arbacia* sperm cells has long been known to deteriorate within 30–60 minutes after dilution in sea water. This so-called "dilution effect" starts with sharp increases in oxygen uptake and rate of movement which presumably rapidly deplete energy stores. Figure 1 illustrates the loss of motility of the FSW-diluted sperm cells, dropping to zero within 40 minutes. The abrupt rise in activity was not evident in these determinations since the first motility rating was not scored until five to six minutes after dilution. In the sperm samples suspended in MBL-ASW a protracted rise in the motility rate occurs that peaks at about 20 minutes and returns to the initial level for the duration of the experiment. When the sperm cells are suspended in a 90:10 mixture of MBL-ASW:FSW, the rate of increase and the maximum rate are both reduced and the sperm cell motility then gradually drops down to about half the speed in the MBL-ASW alone.

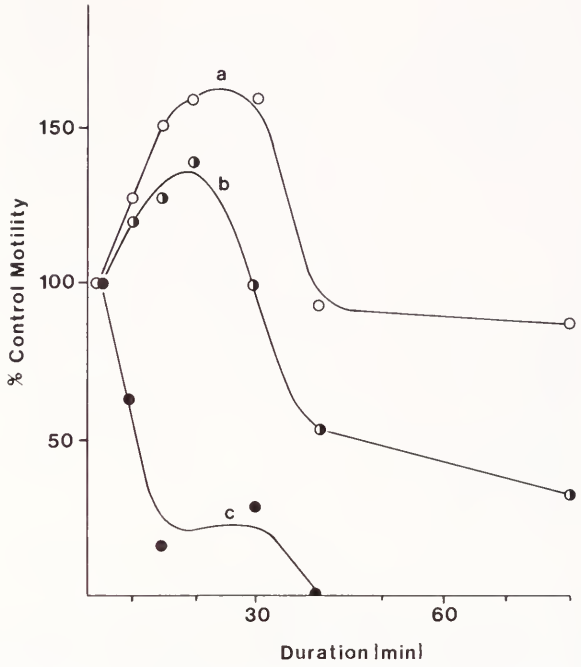


FIGURE 1. Dilution effects: dependence of rate and duration of *Arbacia* sperm swimming on calcium content of the suspension medium: a) open circles, MBL artifical sea water (MBL-ASW); b) half-circles, 90:10 mixture of MBL-ASW:FSW; c) closed circles, filtered sea water (FSW). Ordinate, relative speed of sperm cell progression (as percent of control) following dilution; abscissa, time elapsed after dilution. Sperm cells in MBL-ASW attain higher speeds and endure for longer periods than those in filtered sea water. Temp. 22.5°C.

In previous studies, the immediate effects of several concentrations of calcium and of procaine were examined. The present results with procaine indicate that both time dependence and concentration dependence of the response are modulated by the relative amounts of contaminants (presumably traces of heavy metals) in the medium. Figures 2a (FSW) and 2b (MBL-ASW) show close replication of the respective controls (no procaine) between the duration of sperm cell exposure to filtered sea water and the artificial sea water demonstrated in Fig. 1. In the presence of procaine, the sperm cells in FSW (Fig. 2a) generally undergo a fairly precipitous decline in motility, paralleling the control curve; $10^{-2}$ *M* procaine is predictably inhibitory from the start, the lower concentrations not differing significantly from the controls. With MBL-ASW as the suspending medium (Fig. 2b), even the $10^{-2}$ *M* procaine shows an initial, pronounced, increased acceleratory effect, the swimming speed rising to about 170% of the control rate in 15 minutes. The speed returns to the control levels by 20 minutes and then approaches a plateau while the controls continue their downward rate. Sperm cells suspended in millimolar procaine closely parallel the controls for the first twenty minutes but then decline much less abruptly. The spermatozoa in $10^{-5}$ and $10^{-4}$ *M* procaine, however, peak at nearly double the control speed in ten minutes and decrease gently to a level of forward motion 3–4 times greater than that of the untreated controls.

Procaine acts at the sperm cell surface; purportedly it affects cationic channels involved in calcium entry by displacing calcium from binding sites. Conversely ouabain, a specific inhibitor of $Na^+$, $K^+$-activated, $Mg^{2+}$-dependent adenosinetri-

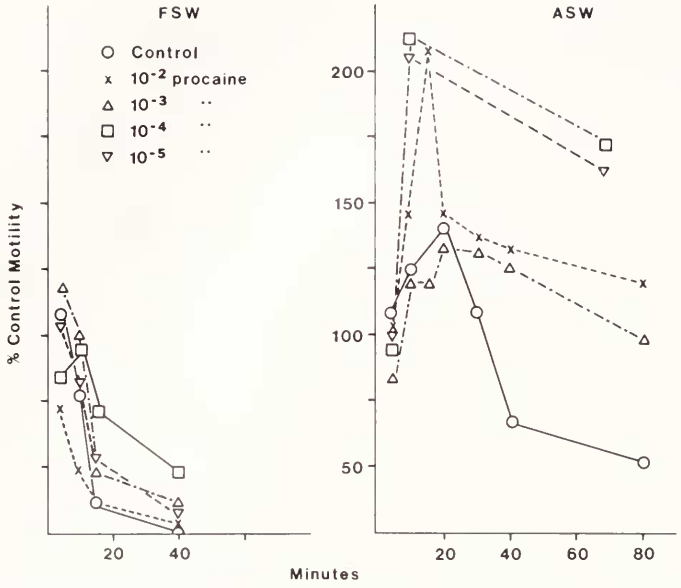


FIGURE 2. Effects of procaine on motility of *Arbacia* sperm suspended in a) FSW and b) MBL-ASW. Ordinate, percent of control motility rate; abscissa, time elapsed following dilution. Note that procaine-treated sperm suspended in MBL-ASW swim at a higher rate of speed for longer periods than do similarly treated sperm in FSW.

phosphatase, is considered to impede $Ca^{2+}$ efflux from cardiac muscle cells (Wood *et al.*, 1972) and may similarly affect sperm cells (*cf.* review, Nelson and McGrady, 1981). The maximum effect of a 10-min incubation in ouabain in filtered sea water occurs at about $10^{-6}$ *M*. This is shown in Figure 3 and confirms the previous report (Nelson, 1972). Increasing the incubation periods in MBL-ASW shifts the maximum response to the left; incubation in $10^{-9}$ *M* ouabain produces a peak in 30 minutes. This is again a dramatic (2.5 fold) increase over that of the initial rate of the FSW controls.

Ouabain toxicity in the mammalian heart cell may be counteracted by the beta-adrenergic-blocking agent propranolol (which in itself exhibits some of the Ca-perturbing properties of a local anesthetic). Propranolol and ouabain were therefore assayed singly and in combination; their interactive effects were tested on the progressive motility of sperm in filtered sea water. Lower concentrations of propranolol have little effect except for a 20% increase at $10^{-6}$ *M* (Fig. 4). However, at 0.1 m*M* a 40% decrease in motility occurs, while the inhibition increases to 80% at 1 m*M*. The peak effect of ouabain alone (at $10^{-6}$ *M*) was a 65% increase in the swimming rate over the controls. In the optimum concentration of ouabain ($10^{-6}$ *M*), increasing amounts of propranolol tend to lower the motility response curve by about 5–10%. Above the optimum concentration of both drugs ($10^{-6}$ *M* each), the ouabain did not significantly influence the response to propranolol. However, with ouabain set a concentration of $10^{-3}$ *M* throughout, the responses to varying amounts of propranolol are markedly altered. Both the prominent peak at $10^{-6}$ *M* and the profound depression at higher propranolol concentrations are eliminated.

Cinchona alkaloids reportedly exhibit digitalis-like properties. Therefore a further test of propranolol in drug-interactive effects on the sperm cell's ability to swim progressively is afforded in the experiments with quinidine. As in the preceding

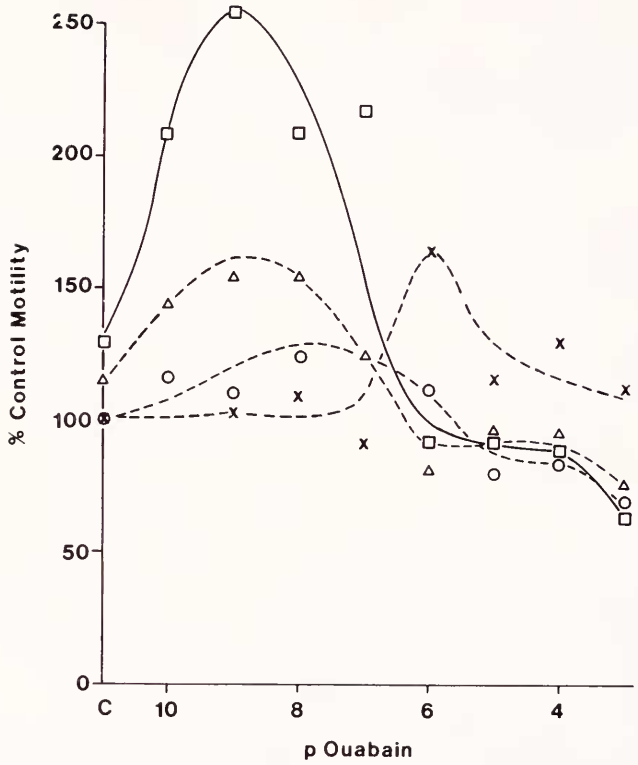


FIGURE 3. Time- and dose-dependent effects of ouabain on *Arbacia* sperm in FSW and MBL-ASW. X's, after 10 min exposure in FSW. Open symbols, in MBL-ASW: circles, 10 min; triangles, 20 min; squares, 30 min after dilution. Ordinate, relative motility in percent of control rate; abscissa, negative log of molar concentration of ouabain in the medium.

experiment (Fig. 4), the peak in $10^{-6}$ *M* propranolol is succeeded by a sharp motility decline with increasing drug concentration. Figure 5a shows that prolonging the incubation in propranolol has little added effect at the lower concentrations, but at micromolar amounts stimulation becomes evident and inhibition is somewhat ameliorated at concentrations of $10^{-5}$ *M* and higher.

Incubation in quinidine alone in FSW (shown in Fig. 5b) over a range of concentrations from $10^{-10}$ *M* to $10^{-4}$ *M* evokes a somewhat uneven but insignificant oscillation around the control rate of movement. The delayed effect on motility does not deviate strikingly from that of the delayed controls when the "dilution" effect is taken into account, *viz.*, a 60% to 80% decrease in progressive movement which is sustained over the entire concentration range after an additional ten minutes of incubation. When the two drugs are tested for interactive effects, the samples incubated in $10^{-3}$ *M* quinidine responded more vigorously than those in $10^{-5}$ *M*. In these experiments, the *Arbacia* sperm cells in seawater suspension were preincubated for five minutes, and, after their motility was rated, to each cuvette was added 0.2 ml FSW in the single treatment labeled "Q" or "P" or 0.2 ml of quinidine for the co-incubation, double-treatment series, labeled "P + Q" in the bar graph diagrams (Figs. 6a & 6b). After the additions, the cuvettes were again inverted 2 times to assure uniform redistribution. The sperm cells were then reoriented centrifugally, and readings were taken at the indicated intervals. Sperm cells exposed

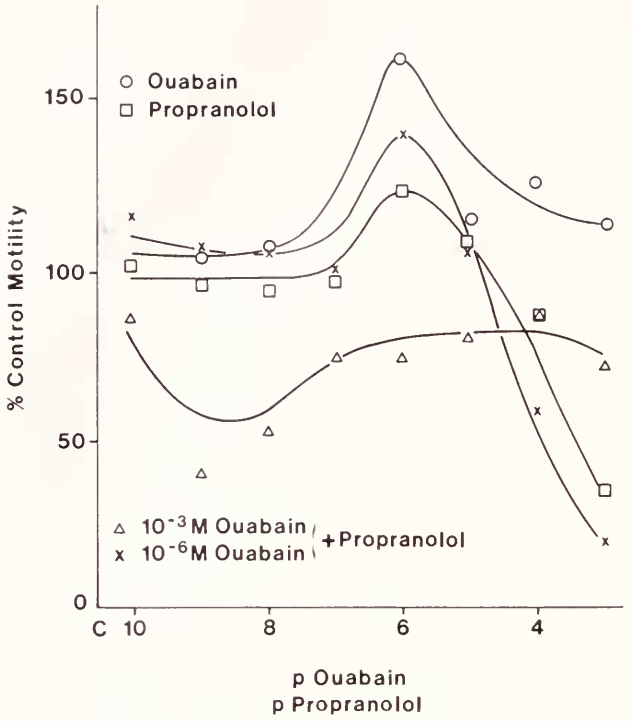


FIGURE 4. Interactive effects following 10-min incubation in the beta receptor blocker propranolol and the cardiac glycoside ouabain on *Arbacia* sperm motility in FSW. Note that the optimum concentration of each drug separately occurs at $10^{-6}$ *M*/l FSW; $10^{-6}$ *M* is also the optimum concentration for both drugs combined. Also note that in the presence of 1 m*M* ouabain, all concentrations of propranolol depressed motility. Ordinate, percent of control progressive motility rate; abscissa, concentrations of the drugs in negative log of molarity.

to $10^{-5}$ *M* quinidine after preincubation in $10^{-4}$ *M* or $10^{-6}$ *M* propranolol (Fig. 6a) show relatively little effect compared to those in $10^{-5}$ *M* quinidine alone.

In marked contrast sperm cells exposed to $10^{-3}$ *M* quinidine following their preincubation in $10^{-4}$ *M* and $10^{-6}$ *M* propranolol respectively, first responded with motility increases, ranging from 160% to 200% of the rates in quinidine alone (Fig. 6b). These bursts of activity were succeeded by precipitous declines to about 50% of the control and quinidine-alone rates on prolonged exposure of the sperm in both cases. In terms of initial reaction to addition of $10^{-3}$ *M* quinidine to sperm cells preincubated in propranolol, the response appears to exceed by far that of a simple addition of the individual response rates.

## DISCUSSION

Membrane-stabilizing agents (local anesthetics and antiarrhythmic drugs) displace calcium from plasma membranes. Perturbation of the calcium not only affects cell permeability (Blaustein and Goldman, 1966) but the drugs, as ligands for $Ca^{2+}$-binding sites, also increase contractile tension in muscle (Bondani and Karler, 1970). Displacement of the calcium required for biological processes may enhance or disrupt flagellar activity.

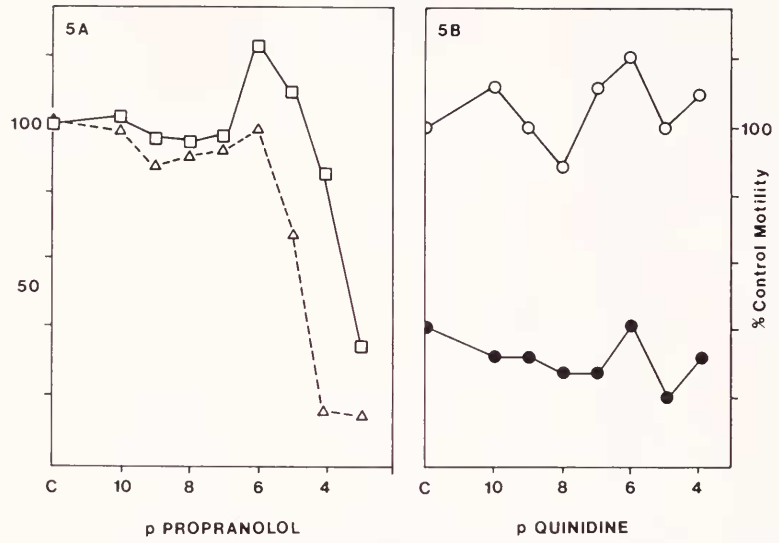


FIGURE 5. Separate effects of varying concentrations of propranolol (A) and quinidine (B) on *Arbacia* sperm in filtered sea water. 5A, triangles, varying concentrations of propranolol without preincubation; square, effects of preincubating in propranolol for 10 min prior to rating motility. 5B, open circles, varying concentrations of quinidine without preincubation; closed circles, same preparation 10 min later, showing that quinidine does not alter the effect of dilution in FSW. Note that while higher concentrations of propranolol markedly depress motile rate, quinidine's effects are fairly constant over the range of concentrations from 0.1 n*M* to 1.0 m*M*. Ordinates, relative motility in percent of control rate; abscissas, drug concentrations in negative log of molar concentration.

Brief exposure to procaine greatly increased *Arbacia* sperm forward motility; however, prolonging the incubation at the same concentration in filtered sea water led to complete cessation of progressive movement (Nelson, 1972). Moreover, EDTA sharply depressed the swimming rate of sea urchin sperm (Young and Nelson, 1974a). The calcium-selective chelator, EGTA, acted similarly at somewhat lower concentrations and sharpened the focus on a critical role for $Ca^{2+}$ (Young and Nelson, 1974b).

Procaine's action was thought to reflect an initial transitory increase in the internal free calcium released from sequestration sites in the membrane; whereas, blockage of the cell's ability to restore calcium to its resting distribution would account for the delayed inhibitory response (Nelson, 1972). These conclusions have been supported by the acceleration and prolongation of motility both in artificial sea water relatively low in heavy metal contaminants, and following procaine treatment in the MBL-ASW. The motility enhancement in MBL-ASW occurs in contrast to the loss of propulsive ability when the spermatozoa are preincubated in FSW solutions of procaine. The immediate response of the sperm cells to procaine in natural sea water resembles the responses observed in synthetic sea water solutions containing only minuscule amounts of heavy metals. If local anesthetics displace calcium from its binding sites in the plasma membrane, then, in the case of sea urchin sperm, at least part of that $Ca^{2+}$ which was released into the cell interior thereby increasing contractile activity was subsequently unable to be restored to physiological levels. In the synthetic salt medium the depressant effects of an excess of intracellular free calcium may be partially alleviated since $Ca^{2+}$ binding sites on the outer surface of the plasma membrane are not occupied by heavy metal ligands.

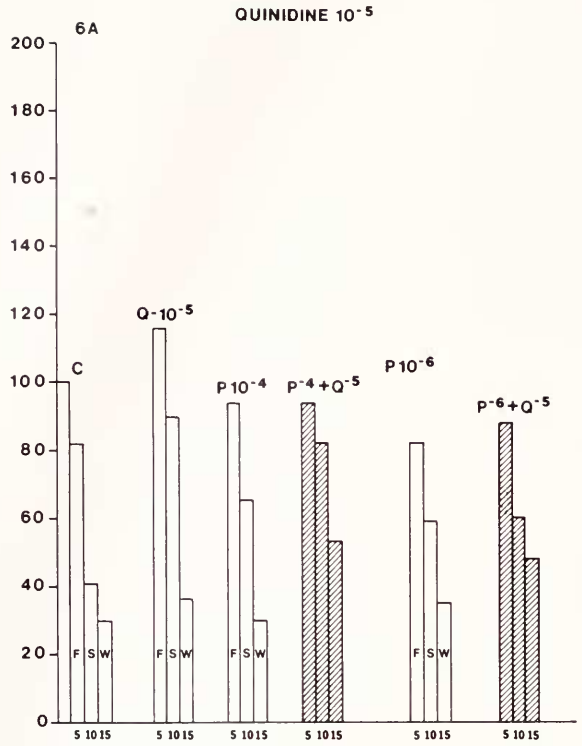


FIGURE 6. Interactive effects of quinidine (Q) and propranolol (P) on progressive movement of *Arbacia* sperm in FSW. Fig. 6A: In all panels, sperm cells were preincubated in FSW for 5 min to establish control rate (100%). At 5 min, 0.2 ml of FSW was added to the sperm suspensions in panel C, Q-$10^{-5}$, P-$10^{-4}$ and P-$10^{-6}$; 10 $\mu M$ quinidine was present in panels Q-$10^{-5}$, $P^{-4} + Q^{-5}$ and $P^{-6} + Q^{-5}$; to panels marked P-$10^{-4}$ and P-$10^{-6}$ propranolol was added to those final molar concentrations. Open bars contained only the one drug indicated (quinidine or propranolol); hatched bars represent cuvettes containing the drug mixtures. Motility ratings were made at three 5-minute intervals. Note that in this series no significant changes in the motile rate were caused by the drugs. Fig. 6B: Similar conditions to those depicted in 6A, except that 1 m$M$ quinidine was tested instead of 10 $\mu M$. In all panels, the sperm cells were preincubated for 5 min in FSW. Then 0.2 ml of FSW was added to the sperm suspensions in the cuvettes represented by the open bars. To the hatched-bar cuvettes was added 0.2 ml of propranolol at a final concentration of $10^{-4}$ $M$ and $10^{-6}$ $M$ respectively. Panels Q-$10^{-3}$, $P^{-4} + Q^{-3}$ and $P^{-6} + Q^{-3}$ contained quinidine, 1 m$M$. Cuvettes represented by open-bar panels contained only quinidine or propranolol alone as indicated. Motility was again rated at 5 min intervals. Note in this series that propranolol alone or in combination with quinidine increased the progressive motion over both the rates of the control and quinidine-alone panels, while in combination, $10^{-3}$ $M$ quinidine plus $10^{-6}$ $M$ or $10^{-4}$ $M$ propranolol, a marked increase in sperm speed occurred.

McGrady (1979) reported that $10^{-3}$ $M$ ouabain significantly depressed the membrane potential of bull sperm and at the same time caused decreases in the frequency and amplitude of the flagellar wave as well as in progressive movement of the cells.

Potentiation of these effects by $10^{-9}$ $M$ ouabain in MBL-ASW suggests that a fine and sensitive balance in the ultimate partition of the calcium across the cell membrane and within the cytoplasmic components must be maintained physiologically and that the presence of heavy metal ions in the environment disrupts the physiological balance.

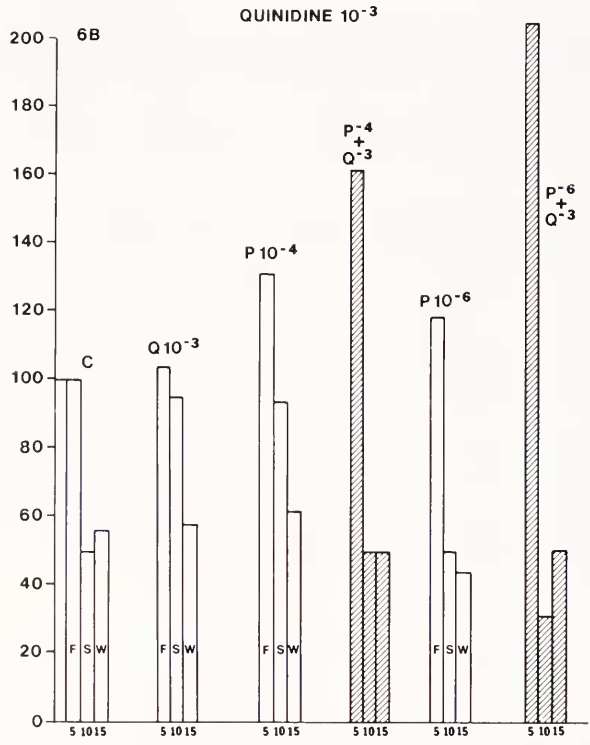


FIGURE 6 (*Continued*)

Ouabain does not compete for $Ca^{2+}$ binding sites, but the cardiac glycoside appears to act on a system involved directly or indirectly in transmembrane $Ca^{2+}$ extrusion. Electron micrographs show cytochemically that, in Ca-loaded, ejaculated bull sperm, ouabain causes the calcium to accumulate at the inner surface of the plasma membrane (Nelson *et al.*, 1980, 1982) as would be predicted on the basis of inactivation of a membrane-sited calcium-extrusion pump. Propranolol antagonizes the effect of ouabain; the degree of interaction depends on the relative concentrations of the drugs. The responses to the two drugs may be ascribed to the differences in sites and modes of their action as indicated above. Both propranolol and quinidine are cardiac antiarrhythmics, although propranolol is a beta-adrenergic receptor blocker and quinidine acts as a blocker of alpha-adrenergic receptors. Quinidine (Fig. 5b) alone caused relatively little change in motility from that of the untreated controls. When $10^{-5}$ *M* quinidine alone was tested there was a 40% increase over the control motility in FSW (Fig. 6a), and when tested after the addition of propranolol, motility remains essentially unaffected. However, when $10^{-4}$ *M* or $10^{-6}$ *M* propranolol (final concentration) was added to the suspensions preincubated in $10^{-3}$ *M* quinidine in FSW, the motility during the first five minutes shot up to 160% and 200% of the control levels, respectively, before dropping back down to the same level as that of the controls during prolongation of the incubation periods.

Rothschild and Tyler (1954) suggested that sperm cells incubated in the chemically defined synthetic medium exhibit greater activity for longer periods by not

being exposed to heavy metal contaminants found in natural sea water. However, the chelating agents EDTA and EGTA exerted only depressant effects on *Arbacia* sperm motility in FSW at all concentrations assayed (Young and Nelson, 1974b). Interference with any of a number of Ca-dependent processes could lead to aberrant behavior. Calcium entry may be restricted by omission or removal (*e.g.* by EGTA) of calcium from the cells' environment. Binding sites on the cell surface may be occupied reversibly or irreversibly by competitive ligands ($La^{3+}$, $Cu^{2+}$, $Pb^{2+}$, $Ni^{2+}$); entry channels may be impeded or inactivated (chelators, $La^{3+}$, anticholinergic agents); calcium may be displaced from sites within the plasma membrane ("membrane-stabilizer"); binding sites on $Ca^{2+}$-dependent enzymes may be occupied by other cations ($Mn^{2+}$, $Zn^{2+}$); calcium extrusion may be inhibited (ouabain or inhibition of enzymes responsible for ATP synthesis). Sparing ATP by inhibition of other ATP-utilizing systems also increases sperm motility.

The time- and dose-dependence of the responses to the transmembrane differential distribution of calcium, as well as to the displacement of calcium ions from binding sites, appear to operate in the case of the sperm cell membrane as in other excitation-effector systems. The plasma membrane is endowed with a variety of receptors with some sites showing affinity for $Ca^{2+}$ which is subject to displacement by membrane-soluble agents; such a membrane exhibits selective ionic permeability and an environmentally sensitive membrane potential.

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